

Registration of 'Brawl CL Plus' Wheat

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ABSTRACT

'Brawl CL Plus' (Reg. No. CV-1074, PI 664255) hard red winter wheat (*Triticum aestivum* L.) was developed by the Colorado Agricultural Experiment Station and released in August 2011 through a marketing agreement with the Colorado Wheat Research Foundation. In addition to researchers at Colorado State University (CSU), USDA-ARS researchers at Manhattan, KS, St. Paul, MN, and Pullman, WA participated in its development. Brawl CL Plus was selected from the cross Teal 11A/'Above'//CO99314 made in 2003 at Fort Collins, CO. Teal 11A is a proprietary hard red spring wheat germplasm line from BASF Corporation that carries a mutant allele at the *Als2* locus (B genome) conferring tolerance to imazamox [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1h-imidazol-2-yl)-5-(methoxymethyl)-3-pyridinecarboxylic acid] herbicide. Above (PI 631449) is a hard red winter wheat cultivar released by CSU in 2001 that carries a mutant allele at the *Als1* locus (D genome) conferring tolerance to imidazolinone herbicides. CO99314 is an experimental line from CSU with the pedigree TX91V4931/'Halt' (PI 584505). Brawl CL Plus was selected as an F_{3,4} line in July 2006 and assigned experimental line number CO06052. Brawl CL Plus was released because it carries two mutations for enhanced tolerance to imazamox herbicide, is adapted under nonirrigated and irrigated production conditions in eastern Colorado, confers moderate resistance to stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks.), and has good milling and bread-baking quality attributes.

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Abbreviations: CSU, Colorado State University; GI, germination index.

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researchers at Colorado State University (CSU), USDA-ARS researchers at Manhattan, KS, St. Paul, MN, and Pullman, WA participated in its development. Brawl CL Plus was selected from the cross Teal 11A/'Above'//CO99314 made in 2003 at Fort Collins, CO. Teal 11A is a proprietary hard red spring wheat germplasm line from BASF Corporation (Florham Park, NJ) that carries a mutant allele at the *Als2* locus (B genome) conferring tolerance to imidazolinone herbicides. Above (PI 631449; Haley et al., 2003) is a hard red winter wheat cultivar released by CSU in 2001 that carries a mutant allele at the *Als1* locus (D genome) conferring tolerance to imidazolinone herbicides. CO99314 is an experimental line from CSU with the pedigree TX91V4931/'Halt' (PI 584505). Brawl CL Plus was selected as an F_{3,4} line in July 2006 and assigned experimental line number CO06052. Brawl CL Plus was released because it carries two mutations for enhanced tolerance to imazamox herbicide, is adapted under nonirrigated and irrigated production conditions in eastern Colorado, confers moderate resistance to stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks.), and has good milling and bread-baking quality attributes.

Methods

Brawl CL Plus was developed using a combination of pedigree and modified bulk-breeding procedures. All early-generation population and line development was done in the greenhouse or at an irrigated field-testing location at Fort Collins, CO. The three-way cross, designated as population X030993, was completed in the greenhouse in spring 2003. In fall 2003, topcross F₁ seeds were vernalized for 8 wk at 2°C

and transplanted into Rootainers (Hummert Intl., Springfield, MO) filled with potting soil. Imazamox herbicide [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-5-(methoxymethyl)-3-pyridinecarboxylic acid; 53 g a.i. ha⁻¹] was applied to the seedlings in a spray chamber at the three-leaf stage. This and all subsequent applications of imazamox herbicide were done with 0.25% (v:v) nonionic surfactant (Activator 90, Loveland Products, Greeley, CO) and 1.5% (v:v) urea-ammonium nitrate fertilizer (UAN 28, Poudre Valley Co-op, Fort Collins, CO). Imazamox-tolerant single-plant selections were made in the greenhouse in February 2004, and the progeny were planted in March 2004 at Fort Collins, CO in short rows as F_{1,2} lines (F₁-derived F₂). Imazamox herbicide (105 g a.i. ha⁻¹) was applied to the rows in May 2004, and a row showing greater uniformity and expression of tolerance (designated as X030993-1) was hand harvested in bulk in July 2004. F_{1,3} seed harvested from this row was planted in an unreplicated field nursery under sprinkler irrigation at Fort Collins, CO in September 2004. In April 2005, the F_{1,3} bulk population was sprayed with imazamox herbicide (175 g a.i. ha⁻¹). In July 2005, the population was subjected to random sampling of approximately 200 spikes at maturity. Spikes were threshed individually and planted in a sprinkler-irrigated headrow nursery in September 2005. Following application of imazamox herbicide (175 g a.i. ha⁻¹) in April 2006, Brawl CL Plus was selected as an F_{3,4} line in July 2006 and assigned the experimental number CO06052.

Brawl CL Plus was evaluated in eastern Colorado in unreplicated preliminary yield trials in 2007, the Advanced Yield Nursery in 2008, the CSU Elite Trial from 2009 to 2011, statewide nonirrigated and irrigated variety trials in 2010 and 2011, the Regional Germplasm Observation Nursery in 2010 and 2011, and the Southern Regional Performance Nursery in 2011. The Advanced Yield Nursery and CSU Elite Trial were arranged in latinized row-column designs with two replications, and the state variety trials were arranged in latinized row-column designs with three replications. Seed purification of Brawl CL Plus was done by headrow progeny purification utilizing DNA marker analysis and a winter seed increase in Yuma, AZ. In summer 2009, 184 single-head selections (F_{3,7} generation) were made from a seed-increase plot of Brawl CL Plus that had been treated with imazamox herbicide (175 g a.i. ha⁻¹). Seed from each head selection was divided between a subsample planted in a progeny row in Yuma, AZ in November 2009, and DNA marker analysis was performed on a subsample using proprietary primer sequences and protocols obtained from BASF Corporation. A bulk tissue sample from at least 11 seedlings of each head selection was used to determine heterozygosity or homozygosity of the F_{3,7} plants for the wild-type and mutant alleles at the *Als1* and *Als2* loci. Based on these tests and on visual observation and removal of off-type progeny rows in Yuma, AZ, 160 progeny rows were bulked to form the breeder seed. Breeder seed was used to plant a 3.6-ha foundation-seed increase under irrigation at Fort Collins, CO in September 2010. The foundation-seed increase was treated with imazamox herbicide

(105 g a.i. ha⁻¹) in April 2011 and rogued for tall variants before harvest.

All statistical analyses were performed with SAS-JMP Pro Version 9.0.2 (SAS Institute, Cary, NC). Agronomic, disease resistance, and end-use quality data were analyzed by the Student's paired *t* test procedure. Yield and grain volume weight data from the CSU Elite Trial and statewide variety trials were subjected to combined analyses of variance across locations and years using a mixed model with genotypes as fixed factors and location-year combinations and replications within location-year combinations as random factors. Only entries common to the trials across all location-years were included. Tukey's Honestly Significant Difference test ($\alpha = 0.05$) was used to compare the least squares means for the genotype effects.

Characteristics

General Description

Brawl CL Plus is an awned, white-glumed, hard red winter wheat. Brawl CL Plus has early maturity, 149.1 d to heading from 1 January, which is 2.1 d earlier than ($P < 0.05$; $n = 60$) 'Hatcher' (PI 638512; Haley et al., 2005), 0.8 d earlier than 'Ripper' (PI 644222; Haley et al., 2007), and similar to ($P > 0.05$) that of Above (148.6 d to heading). Brawl CL Plus is medium-tall (79.0 cm; $n = 130$), 2.8 cm taller than ($P < 0.05$) Hatcher, 1.3 cm taller than Ripper, and 4.6 cm taller than Above. The coleoptile length (evaluated according to Hakizimana et al., 2000) of Brawl CL Plus (78.4 mm; $n = 10$) is longer than ($P < 0.05$) that of Hatcher (69.3 mm), shorter than ($P < 0.05$) that of Ripper (83.3 mm), and similar to ($P > 0.05$) that of Above (78.6 mm). The straw strength of Brawl CL Plus is very good (1.5 score, $n = 17$; 1–9 scale, where 1 = erect and 9 = flat), greater than ($P < 0.05$) that of Hatcher (4.2) and Ripper (2.4 score), and similar to ($P > 0.05$) that of Above (1.8) and 'Thunder CL' (PI 655528; Haley et al., 2009) (1.7). Preharvest sprouting tolerance of Brawl CL Plus, which was assessed through determination of a germination index (GI; Mares et al., 2005) from field-grown samples, is moderate (GI = 0.44; $n = 10$), similar to ($P > 0.05$) that of Hatcher (GI = 0.42) and Ripper (GI = 0.40), and greater than ($P < 0.05$) that of Thunder CL (GI = 0.60) and 'TAM 112' (PI 643143) (GI = 0.64). No objective data are available for the winter hardiness of Brawl CL Plus, but field observations and performance under dry soil conditions during recent winters in Colorado suggest that it is at least adequate for successful production in the central Great Plains region.

Disease and Insect Resistance

Brawl CL Plus has been characterized for disease and insect resistance in Colorado and through cooperative evaluations of the USDA Regional Testing Program. In greenhouse seedling evaluations at St. Paul, MN, Brawl CL Plus was susceptible to moderately susceptible to stem rust races MCCFC, RCRSC, SCCSC, TPMKC, TTKSK, and TTTTF and resistant to moderately resistant to stem rust races QCCSM, QTHJC, RKQQC, and QFCSC, the latter being the most dominant race in the current North American stem rust

population. Field adult-plant evaluations at St. Paul, MN and Njoro, Kenya, have confirmed that Brawl CL Plus is generally moderately susceptible to the North American races of stem rust and to those found in Kenya (*Ug-99* and derivatives). Greenhouse seedling evaluations with leaf rust (caused by *Puccinia triticina* Eriks.) have shown that Brawl CL Plus is susceptible to most common leaf rust races in the United States. (TMGJ, MFPS, MHDS, 10US1-1 MLDS, TNRJ, 10US3-1 TFBJ, and KFBJ) and resistant to race TDBG. Based on these evaluations, Brawl CL Plus was postulated to carry the *Lr14a* leaf rust resistance gene. In 2011, under natural field infection with unknown leaf rust races, Brawl CL Plus showed a susceptible reaction at both St. Paul, MN (50% severity, susceptible infection type) and Castroville, TX (40 to 80% severity, susceptible infection type). Brawl CL Plus carries DNA markers that suggest the presence of *Lr34* (Lagudah et al., 2009; Cao et al., 2010) and *Lr37* (*Ventriup-LN2*; Helguera et al., 2003). In greenhouse seedling evaluations under a low diurnal temperature cycle gradually changing from 4°C at 0200 h to 20°C at 1400 h (Chen and Line, 1995), Brawl CL Plus was susceptible (infection types 8–9 on a 0–9 scale, where 0 = resistant and 9 = susceptible) to races PST-37, PST-45, PST-100, PST-114, and PST-127 of stripe rust (Chen et al., 2010). In greenhouse adult-plant tests under a high diurnal temperature cycle gradually changing from 10°C at 0200 h to 30°C at 1400 h (Chen and Line, 1995; Chen, 2005), Brawl CL Plus was resistant (infection types 2–4) to races PST-100, PST-114, and PST-127. The standard low- and high-temperature profiles were used to simulate early- and late-season growing conditions and to distinguish usable high-temperature adult-plant resistance from all-stage resistance (also called seedling resistance; Chen, 2005). In artificially inoculated field tests at Rossville, KS in 2011, Brawl CL Plus showed a moderate resistant reaction (infection type 5, severity 40–70%; $n = 3$), whereas the susceptible check KS89180B-2 had infection type 9 and 90–95% severity. Field observations of stripe rust severity at Laurel Springs, NC in 2011 were similar to those at Rossville, KS, whereas field observations at four locations in Washington suggested a higher degree of susceptibility to races in that region. Under natural field infection in Colorado in 2010, Brawl CL Plus was moderately resistant (score of 4.4 on 1–9 scale where 1 = resistant and 9 = susceptible; $n = 12$), slightly less resistant than Hatcher (2.3, $P < 0.05$), and more resistant than Ripper (8.3, $P < 0.05$). The susceptibility of seedlings at low temperatures and the resistance of adult-plants in greenhouse and field tests at higher temperatures suggest that Brawl CL Plus has high-temperature adult-plant resistance to stripe rust.

Other evaluations in Colorado or through the USDA Regional Testing Program have shown that Brawl CL Plus is moderately resistant to *Barley yellow dwarf virus* and *Wheat soilborne mosaic virus* and moderately tolerant of acid soils. DNA marker assays suggest that Brawl CL Plus carries *Sbm1* (Perovic et al., 2009). Brawl CL Plus is susceptible to *Triticum mosaic virus*, and its reaction to *Wheat streak mosaic virus* is not known, though it lacks the DNA markers associated with *Wsm1* (Qi et al., 2007) and *Wsm2* (Lu et al., 2012). Brawl CL Plus is susceptible to a collection of endemic biotypes

of the Hessian fly [*Mayetiola destructor* (Say)] (Chen et al., 2009), heterogeneous for resistance to greenbug Biotyp E [*Schizaphis graminum* (Rondani)], and susceptible to Russian wheat aphid (*Diuraphis noxia* Kurdjumov) Biotypes 1 and 2.

Field Performance

Brawl CL Plus was tested at 29 trial locations of the CSU Elite Trial during 2009 (12 locations), 2010 (9 locations), and 2011 (8 locations). In the combined analysis across years, the grain yield of Brawl CL Plus was average (3887 kg ha⁻¹), less than ($P < 0.05$) that of 'Byrd' (PI 664257; Haley et al., 2012a) (4344 kg ha⁻¹), similar to ($P > 0.05$) that of 'Denali' (PI 664256; Haley et al., 2012b) (4027 kg ha⁻¹), Ripper (3987 kg ha⁻¹), and Hatcher (3773 kg ha⁻¹), and similar to the imazamox-tolerant cultivars Above (3737 kg ha⁻¹) and Thunder CL (3579 kg ha⁻¹). In these trials, Brawl CL Plus had above-average grain volume weight (782 kg m⁻³), which was similar to ($P > 0.05$) that of Denali (781 kg m⁻³), Byrd (777 kg m⁻³), and Hatcher (770 kg m⁻³) and greater than ($P < 0.05$) that of Above (766 kg m⁻³), Thunder CL (761 kg m⁻³), and Ripper (754 kg m⁻³).

Brawl CL Plus was tested at 15 trial locations of the nonirrigated Colorado Uniform Variety Performance Trial during 2010 (9 locations) and 2011 (6 locations). In the combined analysis across years, the grain yield of Brawl CL Plus was below average (3603 kg ha⁻¹), less than ($P < 0.05$) that of Byrd (4183 kg ha⁻¹) and Denali (3973 kg ha⁻¹), and similar to ($P > 0.05$) that of 'Settler CL' (PI 653833; Baenziger et al., 2011) (3842 kg ha⁻¹), Hatcher (3789 kg ha⁻¹), Ripper (3725 kg ha⁻¹), Above (3642 kg ha⁻¹), and Thunder CL (3601 kg ha⁻¹). In these trials, Brawl CL Plus had above-average grain volume weight (779 kg m⁻³), which was similar to ($P > 0.05$) that of Denali (785 kg m⁻³), Hatcher (774 kg m⁻³), Byrd (772 kg m⁻³), and Settler CL (771 kg m⁻³) and greater than ($P < 0.05$) that of Above (763 kg m⁻³) and Thunder CL (761 kg m⁻³).

Brawl CL Plus was tested at six trial locations of the Colorado Irrigated Variety Performance Trial (IVPT) during 2010 (three locations) and 2011 (three locations). In the combined analysis across years, the grain yield of Brawl CL Plus was below average (6133 kg ha⁻¹), less than ($P < 0.05$) that of Byrd (6945 kg ha⁻¹) and Settler CL (6133 kg ha⁻¹), and similar to ($P > 0.05$) that of Denali (6581 kg ha⁻¹), Ripper (6514 kg ha⁻¹), Hatcher (6224 kg ha⁻¹), and Thunder CL (6044 kg ha⁻¹). In these trials, Brawl CL Plus had above-average grain volume weight (792 kg m⁻³), similar to ($P > 0.05$) that of Byrd (796 kg m⁻³), Denali (789 kg m⁻³), Thunder CL (784 kg m⁻³), Settler CL (783 kg m⁻³), and Hatcher (782 kg m⁻³) and greater than ($P < 0.05$) that of Ripper (769 kg m⁻³).

Brawl CL Plus was tested in the 2011 Southern Regional Performance Nursery. Averaged across the hard winter wheat region (25 locations), Brawl CL Plus was the 11th-highest-yielding entry in the trial (3549 kg ha⁻¹; 34 total entries).

End-Use Quality

Milling and bread-baking characteristics of Brawl CL Plus and the common check entries were determined using approved methods of the American Association of Cereal

Table 1. Milling, dough-mixing, and bread-baking characteristics of Brawl CL Plus and check entries across multiple evaluations from the 2008, 2009, and 2010 growing seasons in Colorado.

| Trait (unit of measurement) | Samples | Brawl CL Plus | Hatcher | Ripper | Above |
|---|---------|---------------|---------|---------|---------|
| SKCS [†] kernel weight (mg) | 37 | 30.1 | 30.4 ns | 31.6* | 31.3* |
| SKCS kernel diameter (mm) | 37 | 2.71 | 2.63* | 2.70 ns | 2.68 ns |
| SKCS kernel hardness (units) | 37 | 69.2 | 69.3 ns | 67.7 ns | 72.3* |
| Grain protein (g kg ⁻¹) | 35 | 138 | 128* | 134* | 127* |
| Grain ash (g kg ⁻¹) | 35 | 15.1 | 14.7* | 14.7* | 15.1 ns |
| Flour extraction (g kg ⁻¹) | 24 | 672 | 673 ns | 675 ns | 648* |
| Flour ash (g kg ⁻¹) | 34 | 4.4 | 4.4 ns | 4.5* | 4.5* |
| Mixograph peak time (min) | 34 | 4.1 | 4.4* | 3.2* | 2.8* |
| Mixograph tolerance (score) [‡] | 25 | 2.5 | 3.8* | 3.1* | 2.1* |
| Bake mix time (min) | 25 | 3.7 | 4.2* | 3.2* | 2.6* |
| Bake water absorption (g kg ⁻¹) | 25 | 639 | 627* | 639 ns | 615* |
| Loaf volume (L) | 25 | 1.04 | 0.91* | 0.89* | 0.84* |
| Crumb grain (score) [‡] | 25 | 4.5 | 3.8* | 2.8* | 3.4* |

*Significance of the difference between Brawl CL Plus and the check cultivar based on a Student's paired t test procedure at the 0.05 probability level; ns = not significant.

[†]Single-kernel characterization system.

[‡]Scale for mixograph tolerance and crumb grain scores: 6 = outstanding, 0 = unacceptable.

Chemists (AACC, 2000) in the CSU Wheat Quality Laboratory. Multiple location-year samples from the 2008, 2009, and 2010 growing seasons were available to enable comparison between Brawl CL Plus and Hatcher, Ripper, and Above as check entries. The three check varieties have overall good milling properties, whereas the overall baking properties for Hatcher and Ripper are good and Above is poor. Values for milling related variables were generally good for Brawl CL Plus compared with those of the check entries, with comparable kernel characteristics, grain protein and ash concentration, and flour extraction (obtained with the Brabender Quadrumat Senior, C.W. Brabender, South Hackensack, NJ) (Table 1). Values for baking-related variables were generally superior for Brawl CL Plus compared with those of the checks, with comparable dough mixing properties and greater straight-dough pup-loaf volume and crumb grain scores (Table 1). DNA marker assays for high molecular weight glutenin subunits (Butow et al., 2004; Liu et al., 2008) have shown that Brawl CL Plus carries the 2* subunit (*Glu-A1b* allele) at the *Glu-A1* locus, the 7+8 subunits (*Glu-B1b* allele) at the *Glu-B1* locus, and the 5+10 subunits (*Glu-D1d* allele) at the *Glu-D1* locus. Brawl CL Plus does not carry either the T1BL-1RS or T1AL-1RS translocation.

Availability

Brawl CL Plus contains two patented herbicide tolerance traits owned by BASF Corporation that confer tolerance to imidazolinone herbicides, such as imazamox. Any use of Brawl CL Plus requires a Material Transfer Agreement (for research use only) or a commercial license to the traits, as well as permission from the originator (CSU). Seed requests should be sent to the corresponding author, who will forward the request for seed to BASF Corporation. No seed will be distributed for 20 yr from the date of release without written permission from both BASF and CSU. Seed of Brawl CL Plus has been deposited with the National Plant

Germplasm System, where it will be available for distribution by the NPGS upon expiration of the patents or 20 yr after the date of publication.

The Colorado Agricultural Experiment Station will maintain breeder seed of Brawl CL Plus. Multiplication and distribution rights of other classes of certified seed have been transferred from the Colorado Agricultural Experiment Station to the Colorado Wheat Research Foundation, 4026 South Timberline Road, Suite 100, Fort Collins, CO, 80525. Brawl CL Plus has been submitted for U.S. Plant Variety Protection

(PVP) under Public Law 91-577 with the Certification Only option. Recognized seed classes will include the Foundation, Registered, and Certified seed classes.

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